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Impact of physical and chemical pre-treatments on *in vitro* seed germination of brinjal (*Solanum melongena* L.)

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Abstract

The pre-treatment of seeds with physical and chemical agents or exposure to different temperatures improves the viability and germination of seeds. The current study was aimed to explore the consequence of various pre-treatments on *in vitro* seed germination percentage of brinjal (*Solanum melongena* L.) var. Mattu Gulla. The seeds were pre-treated with Helium-Neon (He-Ne) laser (632.8 nm, 7.1 mW with a power density of 4.0198 mW/cm^2), low and high temperatures, acid and alkali. The pre-treated seeds were surface sterilized and germinated aseptically on Murashige and Skoog medium and incubated at 25±2 ºC with 16 h photoperiod. A positive effect of the pre-treatments on germination percentage was observed for He-Ne laser (30 J/cm^2), low temperature (4 and -20 °C) and sulphuric acid (30 %). The low temperature pre-treatments were significantly improved the germination of Mattu Gulla seeds while alkali pre-treatment with potassium hydroxide (2.5, 5.3 and 7.6 M KOH) showed inhibitory effect on *in vitro* seed germination. Thus, the study revealed the integral role of pre-treatment especially with low level laser irradiation and low temperature treatment for the augmented *in vitro* seed germination of brinjal variety.

Key words: Brinjal, Mattu Gulla, exposure, treatment, *in vitro*, seed germination, comparative analysis.

Introduction

The brinjal (*Solanum melongena* L.), is an economically and medicinally important vegetable crop (Kalloo and Bergh, 1993). It is a virtuous source of minerals, vitamins, and antioxidants making it a nutritionally valuable for human consumption (Urquiaga and Leighton, 2000). The resources and germplasm collections of brinjal have been well documented, analyzed and conserved worldwide (Sarathbabu *et al.,* 1999) exhibiting broad range of variation in morphological and biochemical characteristics (Arivalagan *et al.,* 2012). The germplasm collections have been classified into 11 groups and 475 cultivars worldwide, based on distinctive features and taxonomic methods (Martin and Rhodes, 1979).

Seeds are the primary source for the sustainable production of many agriculturally important crops grown all over the world such as cereals, edible oils, pulses, and livestock fodders. The external (light, temperature, moisture) and internal factors (abscisic acid, gibberellic acid, reactive oxygen species, hydrogen cyanide, nitrous oxide, and alcohols) regulate the seed dormancy and germination process (Bewley and Black, 1994). The vegetable crops have orthodox type of seeds, and show variation in seed germination owing to genetic composition, aging, dormancy and loss of viability through postharvest storage. Diverse treatments such as scarification, stratification and exposure/treatment of various physical and chemical agents are extensively used to improve the seed germination rate of several important crop and medicinal plants (Bone, 2003; Brady and McCourt, 2003; Vasilevski, 2003). Currently, number of physical (temperature, light, laser, magnetic and microwave radiation) and chemical agents (acid, alkali, plant growth regulators) are commonly used in pre-sowing treatments, some of which have resulted in rapid and uniform seed germination in maize (Samuilov and Garifullina, 2007), radish (Muszyñski and Gladyszewska, 2008), endive and chicory (Tzortzakis, 2009), barley seeds (Dymek *et al.,* 2012) and sugar beet (Prooeba-Bialczyk *et al.,* 2013).

The dormancy and delayed germination rate have been reported in diverse species of *Solanum* (Adebola and Afolayan, 2006; Taab and Andersson, 2009) comprising several varieties of *S. melongena* and interrelated species (Joshua, 1978; Demir *et al.,* 2005). The germination percentage in *S. incanum*, *S. torvum*, *S. integrifolium, S. surattense*, *S. khasianum*, *S. sanitwongsei* and hybrids of *S. melongena* x *S. integrifolium* was reported from 15-50 % (Joshua, 1978). Mattu Gulla is a distinctive variety of brinjal propagating specifically in Mattu village, Udupi District, Karnataka, India since 500 years. The fruit is green in colour, ovoid in shape with white stripes and thorn in the stalk (Bhat and Madhyastha, 2007). This distinctive variety was tagged with the Geographic Indication (GI) Status provided by Geographical Indications Registry (GIR), India (Geographical Indications Journal 2011). Mattu Gulla shows 60-70 % germination in every season (personal communication by farmers) and farmers are losing significant percentage of seedlings every year due to fungal disease, nematode infection and unseasonal rains during nursery development. Consequently, the current study was intended to test the influence of pre-treatments or priming with laser, low and high temperatures, acid and alkali on the *in vitro* germination responses of Mattu Gulla seeds.

Materials and methods

The mature and ripe fruits of brinjal (*Solanum melongena* L.) var. Mattu Gulla were collected from Mattu village in Udupi District of Karnataka, India. The seeds were detached physically from the ripe fruits, the mature and uniform seeds were selected, dried under shade and stored at room temperature (~ 28-30 °C) for further experiments. The vigorous and identical seeds were saturated in distilled water for 3 h prior to pre-treatment. Each group comprised of 25 seeds in triplicate and the experiments were accomplished thrice. Seeds were exposed to different doses of He-Ne laser (20, 25 & 30 J/cm^2) as described by Muthusamy *et al.* (2012), low temperatures (4, -20 and -80 °C for 0, 1, 3, 6, 9, 12 and 24 h), high temperature (30, 35 and 40 °C for 0, 5, 10, 15, 20, 25 and 30 min in water bath), acid (30, 40 and 50 % H_2SO_4 for 0, 2, 4, 6, 8, 10, 12 and 14 min) and alkali concentrations (2.5, 5.3 and 7.6 M KOH for 0, 2, 4, 6, 8, 10, 12 and 14 min). The unexposed/treated seeds were considered as control groups.

The pre-treated seeds were surface sterilized separately by washing the seeds with tap water for 3 times, soaked in soap solution containing Tween 20 for 5 min, trailed by treatment with 70 % ethanol for 1 min, 0.1 % (w/v) disinfectant for 5 min and finally seeds were rinsed 5 times with sterile distilled water. The disinfected seeds were inoculated on MS (Murashige and Skoog, 1962) basal medium with sucrose (30 g L^{-1}), 0.8 % (w/v) agar and adjusted to pH 5.8 before autoclave, and incubated in dark at 25±2 ºC for 24 h for *in vitro* germination. After 24 h, the seeds in the culture bottles were transferred to light under cool-white fluorescent tubes (40 µMol m^{-2} s^{-1}) with 16 h photoperiod. The germination percentage of pre-treated and control seeds were recorded for 10 days and calculated for percentage of germination according to Coolbear *et al.* (1984) and modified by Farooq *et al.* (2005). The data on percentage of *in vitro* germination were subjected to Duncan's new multiple range test (DNMRT) and compared at $P = 0.05$.

Results and discussion

The seed priming has become a regular exercise to advance the seed performance as well as to enhance the rate of uniformity in seed germination and further development of seedlings under various field conditions during last three decades. The proportion of germination was substantially higher in the He-Ne laser irradiated groups compared to control group. The 30 J/cm^2 He-Ne laser exposed seeds revealed significantly, the highest percentage of germination with 97 % rate followed by 25 and 20 J/cm^2 seeds (Fig. 1). Similarly, the seeds were subjected to cold treatment at 4, -20 and -80 ºC for 0 to 24 h and observed the highest germination percentage for 1 and 2 h at 4 ºC and -20 ºC pre-treatment than 0 h (control) treatment and was found to be significant according to DNMRT analysis (Fig. 2). The germination percentage decreased from 3, 4 and 6 h treatments at 4, -20 and -80 ºC but

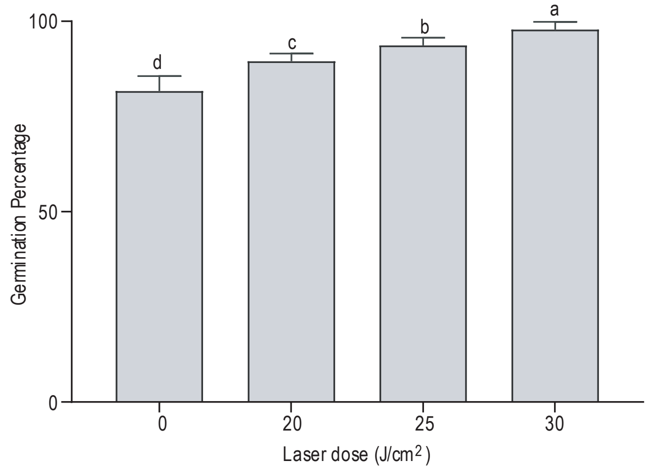

Fig. 1. Effect of He-Ne laser irradiation on seed germination percentage of brinjal (*Solanum melongena* L.) var. Mattu Gulla. Mean values with the same alphabet are not significantly different (P= 0.05) according to DNMRT. Data are the means and standard deviation of the mean for n = 3 independent experiments.

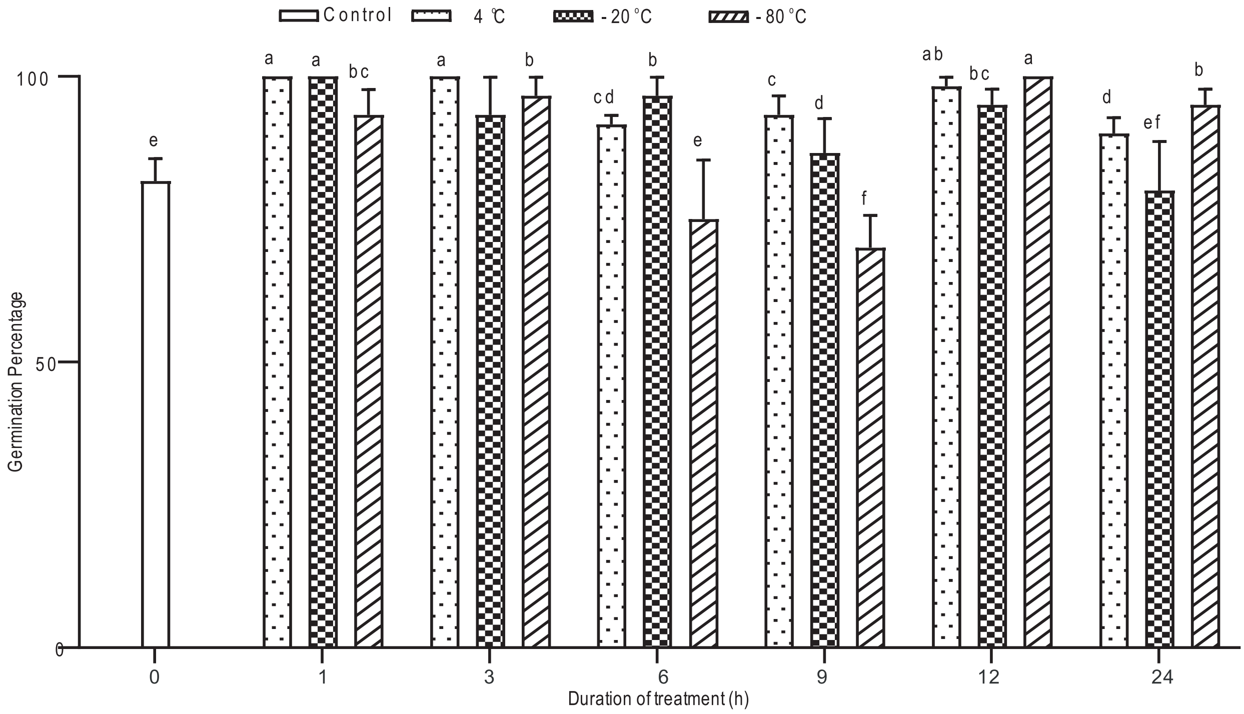

Fig. 2. Effect of cold pre-treatment on seed germination percentage of brinjal (*Solanum melongena* L.) var. Mattu Gulla. Mean values with the same alphabet are not significantly different (P= 0.05) according to DNMRT. Data are the means and standard deviation of the mean for n = 3 independent experiments.

statistically not significant. Among the different hours of -80 ºC pre-treatment, the 2, 5 and 6 h of incubation showed highest germination percentage followed by 3 and 4 h treatment. The 1, 2, 5 and 6 h pre-treatment at -80 °C showed over 90 % germination whereas 3 and 4 h pre-treatment lead to decreased germination percentage (Fig. 2).

In the present study, low level laser irradiation showed significant increase in percentage of seed germination. Present observations are in conformity with 20-35 % enhanced seed germination by Li and Feng (1996) in *Atractylodes macrocephala*. In earlier studies, the improved seed germination and days to 50 % germination was observed in brinjal by laser treatment (Muthusamy *et al.,* 2012). Stimulatory effect on germination percentage of *Amaranthus* and other vegetables, maize, wheat was observed with varied intensity of laser irradiation (Chen, 2008). Furthermore, substantial stimulatory effects on germination of seeds with low power He-Ne laser irradiation was noted by Perveen *et al.* (2010) and suggested that enhancement of seed germination are due to potential variations in physio-biochemical state of seeds and plantlets. Similarly, Evenari (1965) have proposed the dormancy breaking properties of He-Ne laser irradiation even for slow germinating seeds.

Results of present study are corroborated with seed priming treatments with moist-chilling (Van Staden *et al.,* 2006), cold storage (De Silva and Peiris, 1997) and low temperatures (Bian *et al.,* 2013; Demir and Mavi, 2004) which showed enhanced percentage of seed germination. During cold stratification (2-5 ºC), the relative enhancement in growth promoting substances including gibberellic acid was noted in both seed coat and embryo which promotes the seed germination (Yang *et al.,* 2008). However, observations of present study showed significant improvements in seed germination at 4 and – 20 °C for 1-2 h treatments.

There was no improvement in germination percent of with the pre-treatment of 30, 35 and 40 ºC. In 30 and 35 ºC, the germination percent was almost similar with reference to un-treated control whereas, it showed decreasing trend at 40 ºC treatment compared to un-treated control. The warm temperature pre-treatments for 20 min at 40 °C showed only 40 % germination whereas 25 min treatment showed 65 % of germination, respectively (Fig. 3). Khan and Rizvi (1994) reported the stimulation in germination percentage in light and cooler alternating temperatures (25:10 ºC) and decreased trends was noted in warmer temperatures (30:15; 30:20; 35:25 ºC). Improved seed vigor was established in eggplant and chilli with hydropriming for 48 h and sand matric priming with 80 % water holding capacity (Venkatasubramanian and Umarani, 2007). Similarly, the matric priming was noted to be the best among other priming techniques for okra whereas hydropriming was most suitable methods for beet root (Nirmala and Umarani, 2014).

The percentage of seed germination increased slightly in 2, 4, 6 and 8 min treatment with 30 % H_2SO_4 whereas 10 and 12 min pre-treatment showed no difference from control, and least percentage of germination was noted with 14 min pre-treatment with 30 % H_2SO_4. An enhancement in percentage of germination was noted with 6 min pre-treatment of 40 % H_2SO_4 whereas slight reduction at 2 and 4 min as well as enhancement at 8, 10, 12 min was noted. Similarly, insignificant increase in germination was noted at 2-10 min pre-treatment with 50 % H_2SO_4 whereas decreasing trend was noted at 12 and 14 min treatment. Lowest percentage of germination was noted at 14 min pre-treatment in all three concentrations of H_2SO_4 (Fig. 4). The present study is in conformity with Ramezani *et al.* (2010) wherein maximum percentage of germination was observed with H_2SO_4 pre-treatment for 10 min. The pre-treatment with different acid concentration (H_2SO_4) helps in rupturing the seed coat and facilitate the seed germination process. Similar results were reported in *Daucus*

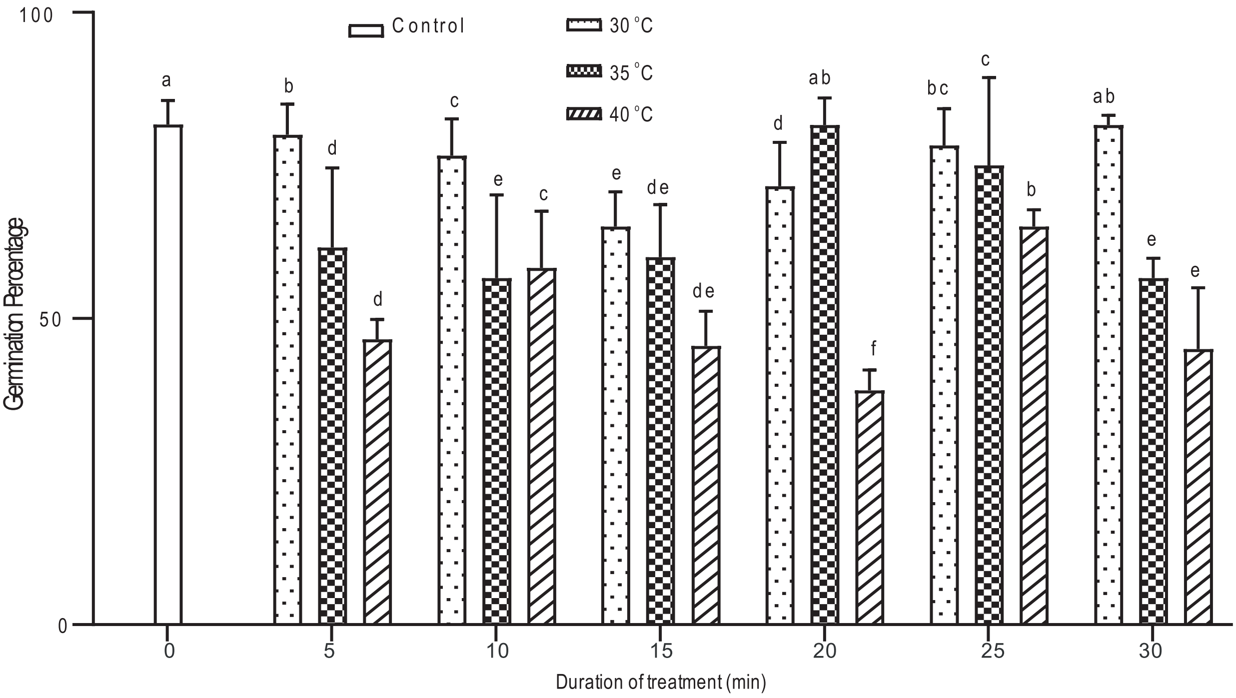

Fig. 3. Effect of temperature pre-treatment on seed germination percentage of brinjal (*Solanum melongena* L.) var. Mattu Gulla. Mean values with the same alphabet are not significantly different (P= 0.05) according to DNMRT. Data are the means and standard deviation of the mean for n = 3 independent experiments.

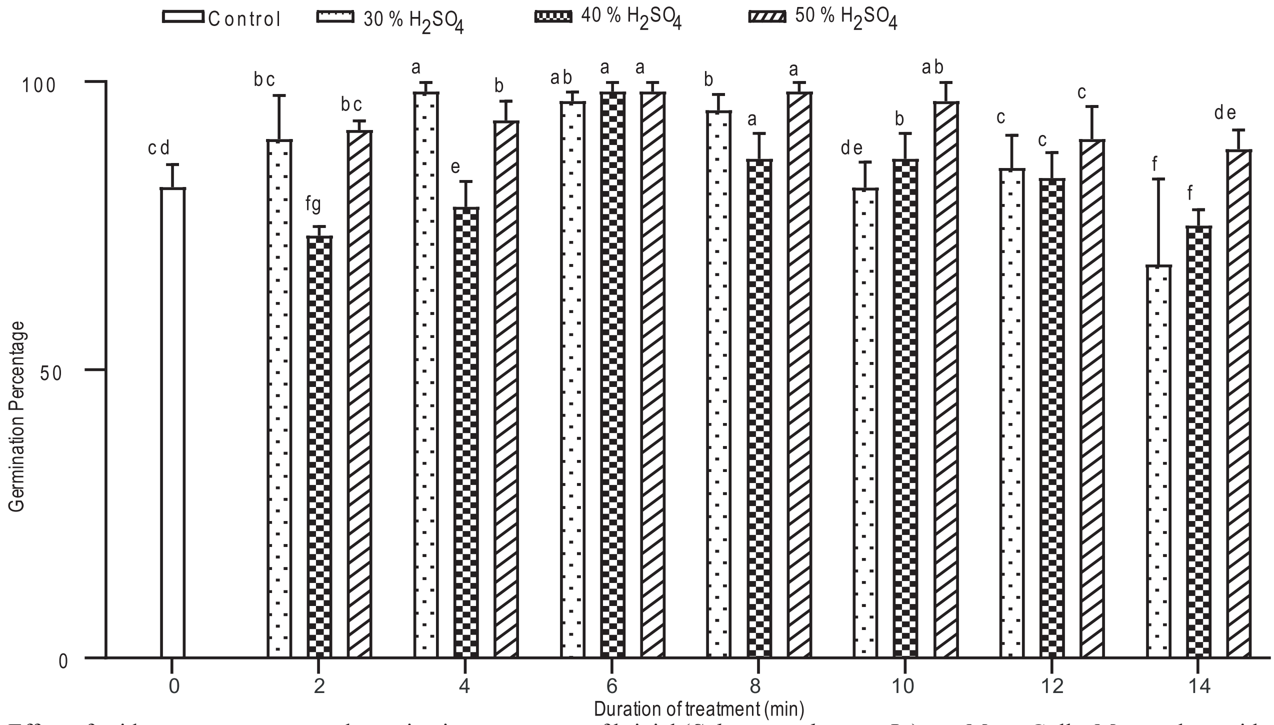

Fig. 4. Effect of acid pre-treatment on seed germination percentage of brinjal (*Solanum melongena* L.) var. Mattu Gulla. Mean values with the same alphabet are not significantly different (P= 0.05) according to DNMRT. Data are the means and standard deviation of the mean for n = 3 independent experiments.

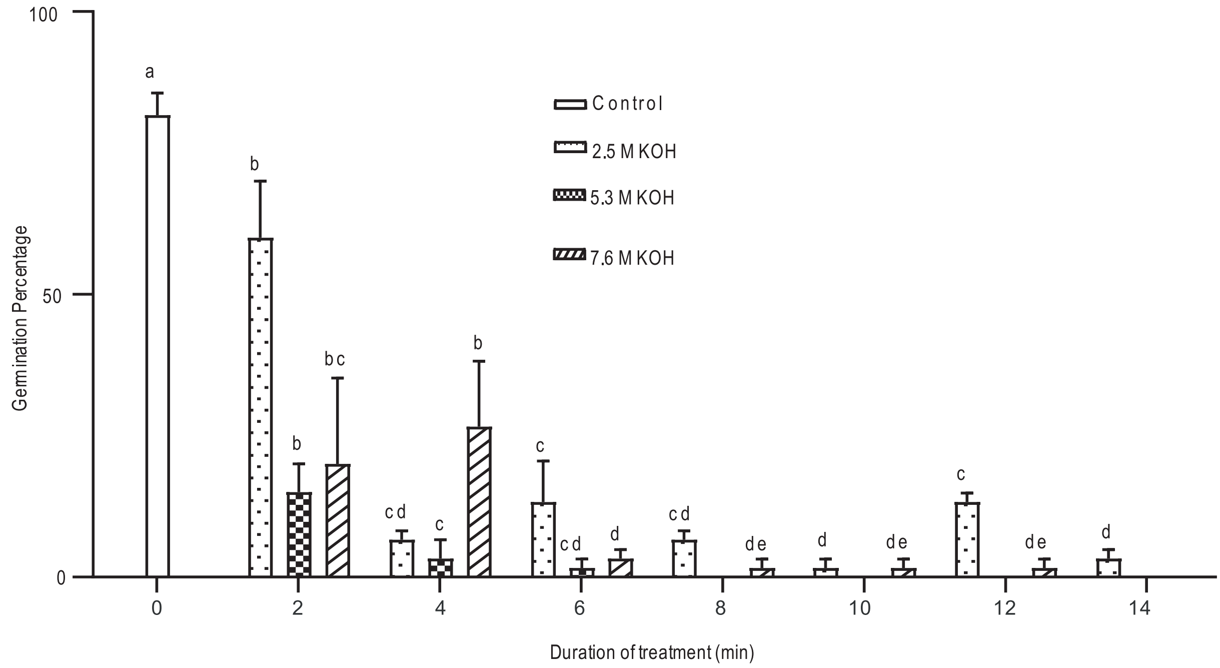

Fig. 5. Effect of alkali pre-treatment on seed germination percentage of brinjal (*Solanum melongena* L.) var. Mattu Gulla. Mean values with the same alphabet are not significantly different (P= 0.05) according to DNMRT. Data are the means and standard deviation of the mean for n = 3 independent experiments.

carota L., *Brachiaria brizantha*, *B. decombuns* and *Panicum antidotale* (Bralewski *et al.,* 2004), *Tamarindus indica, Retama raetam, Ononis serrata* and *Mesembryanthemum crystallinum* (Muhammad and Amusa, 2003).

Among three concentrations of KOH, only 60 % germination was noted at 2.5 M KOH for 2 min and reduced drastically from 4 to 14 min treatments of 2.5 M KOH. In 5.3 and 7.6 M KOH, only 15 and 20 % germination was recorded even at 2 min treatments and further it was reduced bare minimum or it shows no germination (Fig. 5). In the present study, drastic reduction in germination percentage was noted in three concentrations of KOH treatments. Comparable results were reported by Ramezani *et al.* (2010) with reduction of germination percentage by 76 % NaOH and in brinjal (Shahlaei *et al.,* 2009). Jett *et al.* (1996) have suggested that increased availability of oxygen during pre-treatment and decreased electrolyte leakage during imbibition are two key factors which facilitate enhanced seed germination. Recently, Ling *et al.* (2014) showed the stimulatory effects of cold plasma treatment on mobilization, utilization efficiency and percentage of diminution of seed reserved energy, which lead to enhanced germination and seedling growth of soybean seeds. The change in temperature was noted using infrared camera following laser irradiation at 650 nm and the increased temperature, which triggers seed metabolism for germination (Aguilar *et al.,* 2015). Similarly, the ephemeral increase in temperature which may be absorbed by the biomolecules and activate or inhibit the enzymes of seed germination (Rassam 2010). Recently, Macovei *et al.* (2016) reviewed the pre-germinative metabolism from physio-biochemical, genetical and omics studies performed in model as well as crop plants to understand the strategy to manipulate important seed traits. The present study demonstrates utility of pre-treatments to improve the germination percentage of brinjal seeds (var. Mattu Gulla) especially with He-Ne laser and low temperature, as it may be useful for the breeding purposes and in conservation of seed as germplasm resources.

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